
Strategic actions to prevent the entrance and early detection of Foc TR4 in Latin American and the Caribbean



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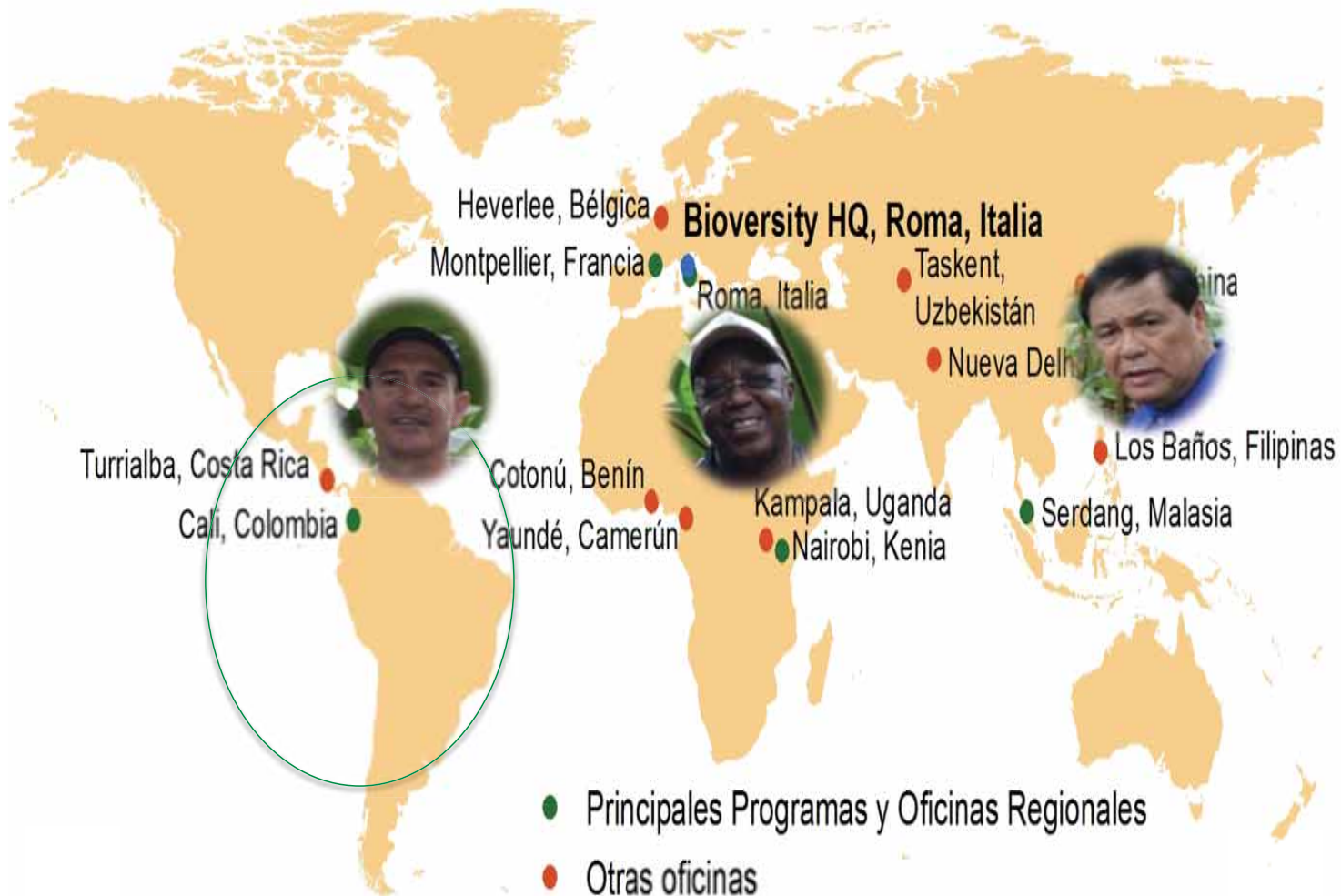
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Costa Rica, Julio, 2013



Where we are?

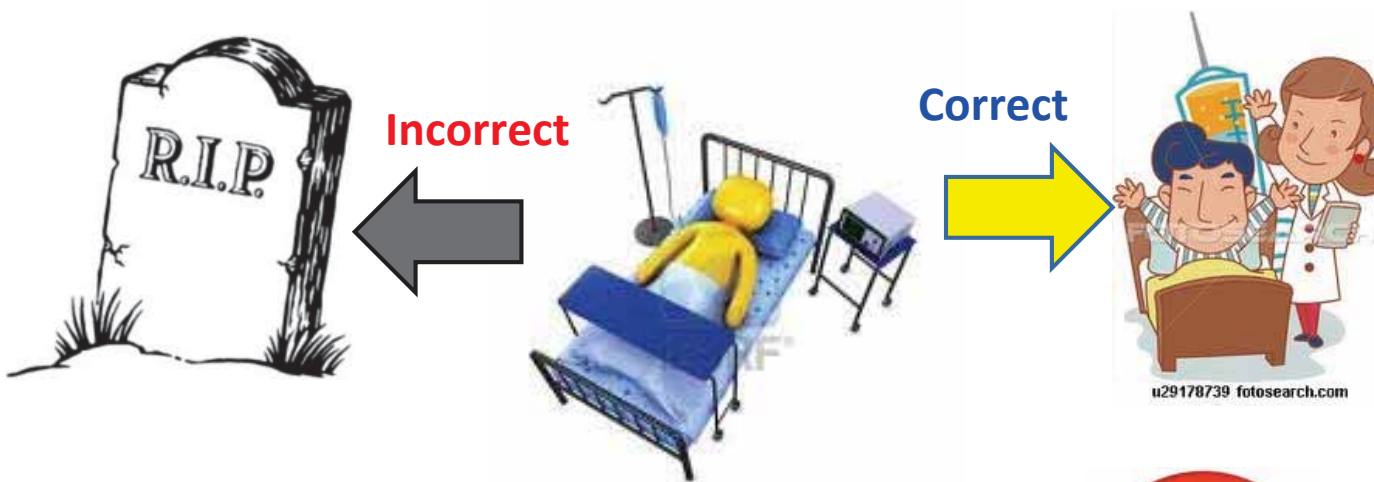


Research Lines, Objectives and Targets – Bioversity LAC

RESEARCH LINES	OBJECTIVES	RESEARCH TARGETS
<p>➔ Risk Assessment and Surveillance of key banana diseases</p>		
<p>Use of beneficial microbial diversity for disease management and enhancing productivity</p>		
<p>Soil health for plant health and enhancing productivity</p>		
<p>Epidemiology for Integrated Management of Diseases</p>		
<p>Socioeconomic and impact assessment along the value chain</p>		



DIAGNOSTIC OF PATHOGENS



Is this different in Agriculture?



Even worst for quarantine di\$easeS - Foc TR4

Races of Foc in banana



R1 – 1890
VCG0120 -01224

Gross Michel



Bluggoe
Cooking bananas



R2 ~ 1900 also affects R1 susceptibles
VCG0120, -8

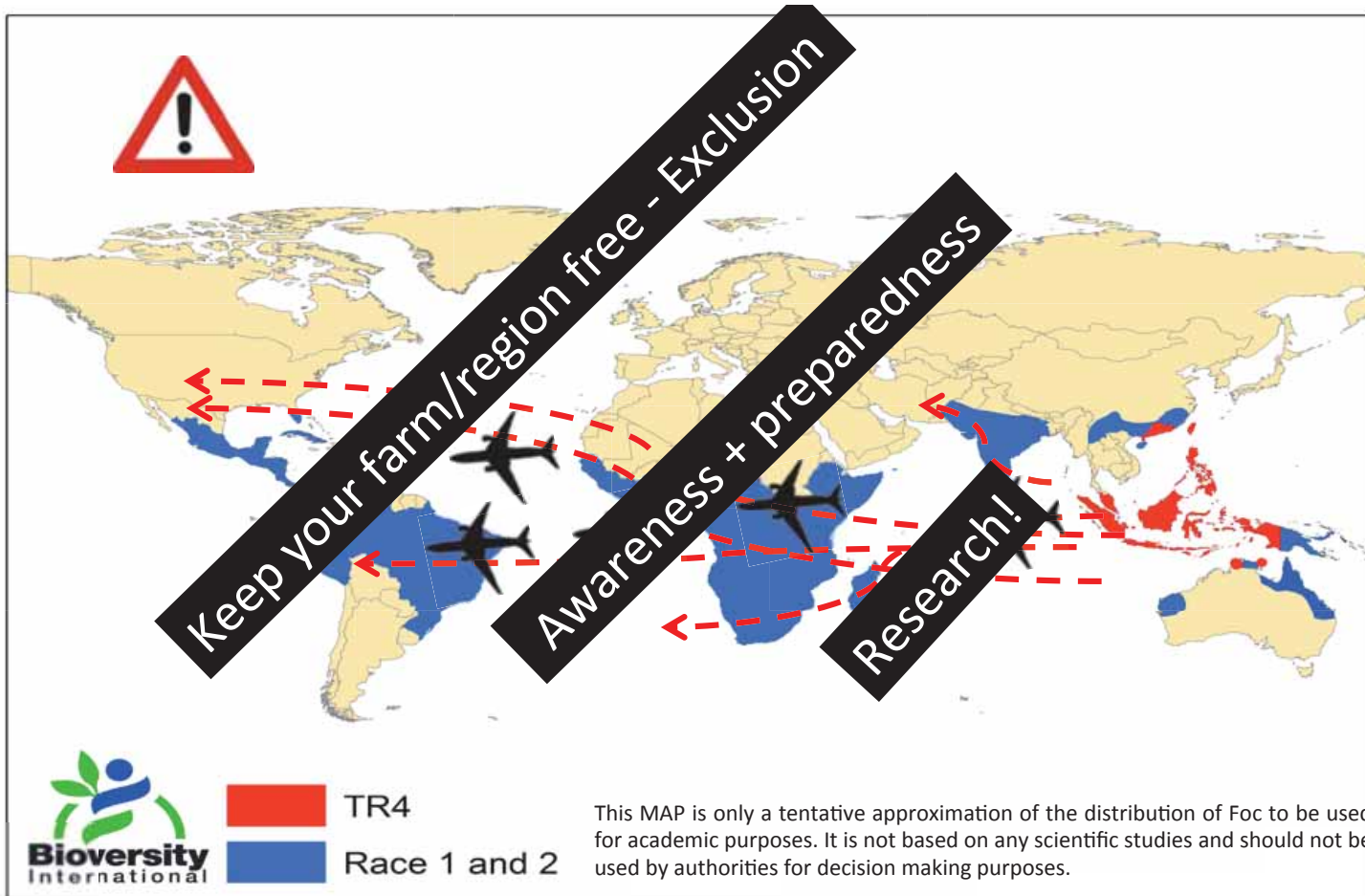


R4

ST4 ~ 1967 – Subtropical areas
Related to no favourable conditions – **cold**
VCG- 0120,-1,-2,-9,-10,-11,-15

TR4 ~1990's – Tropical areas
Both tropical and subtropical conditions
VCG01213, (01213/16 , -16)

Fusarium Wilt of Banana: Global distribution & 3 Urgent Actions



****Los síntomas provocados por raza 4 Tropical son similares a los de la raza 1 y 2**



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Raza 4T –Cavendish ,Taiwan



© Miguel Dita

Raza 1 – Gros Michel, Costa Rica

****Los síntomas provocados por raza 4 Tropical son similares a los de la raza 1 y 2**



Raza 4 tropical – Cavendish, Austral

Cortesía Wayne O'Neil - Australia

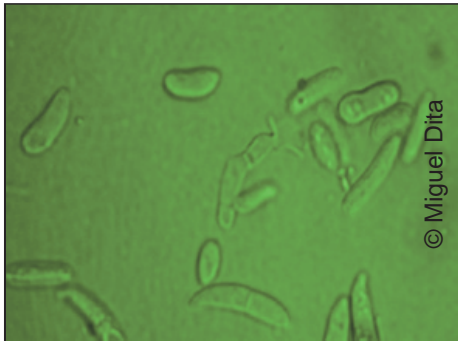


Raza 2 – Monthan (ABB), Brasil

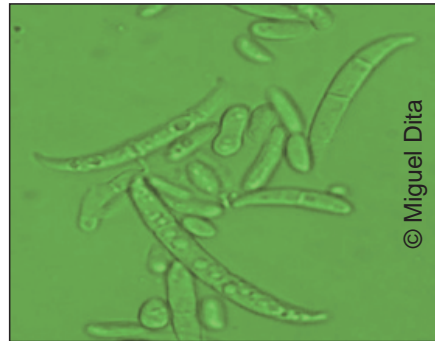
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A diagnostic tool for Foc ?

Structures of *F. o f. sp. cubense*



Microconidia are 5 - 16 x 2.4 - 3.5 μm , one- or two-celled, oval- to kidney-shaped, and are borne in false heads



Macroconidia: are 27 - 55 x 3.3 - 5.5 μm , four- to eight-celled and sickle-shaped with foot-shaped basal cell

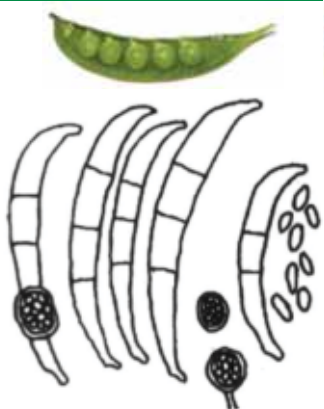


Chlamydoconidia: Terminal and intercalary are 7 - 11 μm in diameter, usually globose and are formed singly or in pairs in hyphae or conidia

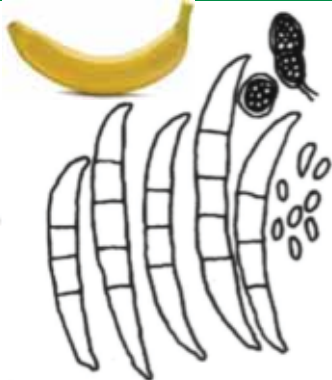
- Fox: ~ 100 *formae speciales* cause wilting in plants
It contains pathogenic and saprophytic **strains that cannot be distinguished morphologically**

Source: Ploetz (2000)

Foc cannot be distinguished morphologically from other Foxy's



f. sp. pisi race2
from peas (Illinois)



f. sp. cubense race4
from bananas (Taiwan)



f. sp. vasinfectum race1
from cotton (California)



saprophyte
from soil (California)



f. sp. pisi race5
from peas (Washington)



f. sp. cubense race4
from bananas (Philippines)



f. sp. vasinfectum race3
from cotton (Israel)



f. sp. lini
from flax (Minnesota)

Source: Smith (2007)

Development of a molecular marker for specific detection of *Fusarium oxysporum* f. sp. *cubense* race 4

ST4 + TR4

Ying-Hong Lin · Jing-Yi Chang · En-Tzu Liu ·
Chih-Ping Chao · Jenn-Wen Huang ·
Pi-Fang Linda Chang

Received: 24 January 2008 / Accepted: 28 August 2008
© KNPV 2008



Plant Pathology (2010) 59, 348–357

Doi: 10.1111/j.1365-3059.2009.02221.x

A molecular diagnostic for tropical race 4 of the banana fusarium wilt pathogen

Only TR4

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Eur J Plant Pathol
DOI 10.1007/s10658-012-0096-0

**A molecular diagnosis method using real-time PCR
for quantification and detection of *Fusarium oxysporum*
f. sp. cubense race 4**

Ying-Hong Lin • Ching-Chung Su •
Chih-Ping Chao • Chi-Yu Chen •
Chung-Jan Chang • Jenn-Wen Huang •
Pi-Fang Linda Chang

Accepted: 10 September 2012

Eur J Plant Pathol (2013) 135:903–911
DOI 10.1007/s10658-012-0136-9

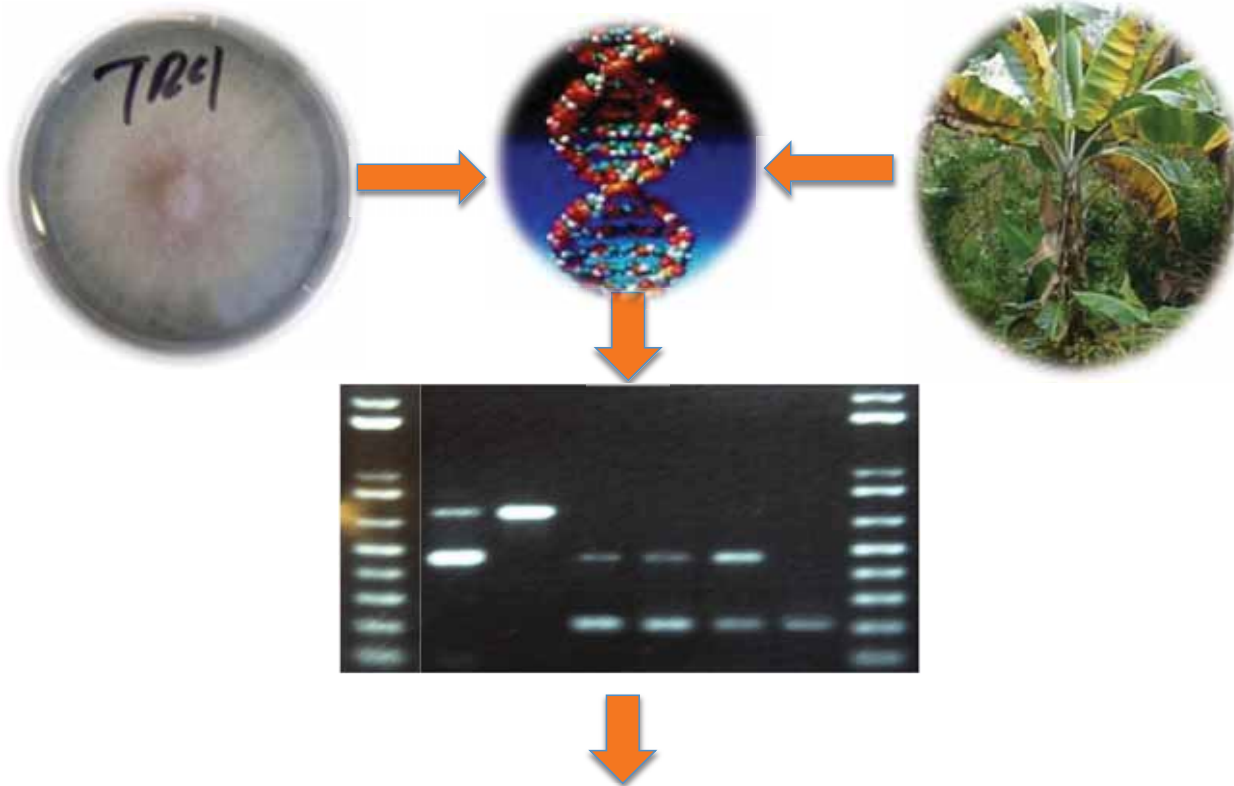
**Development of a loop-mediated isothermal amplification
assay for rapid and sensitive detection of *Fusarium*
oxysporum f. sp. *cubense* race 4**

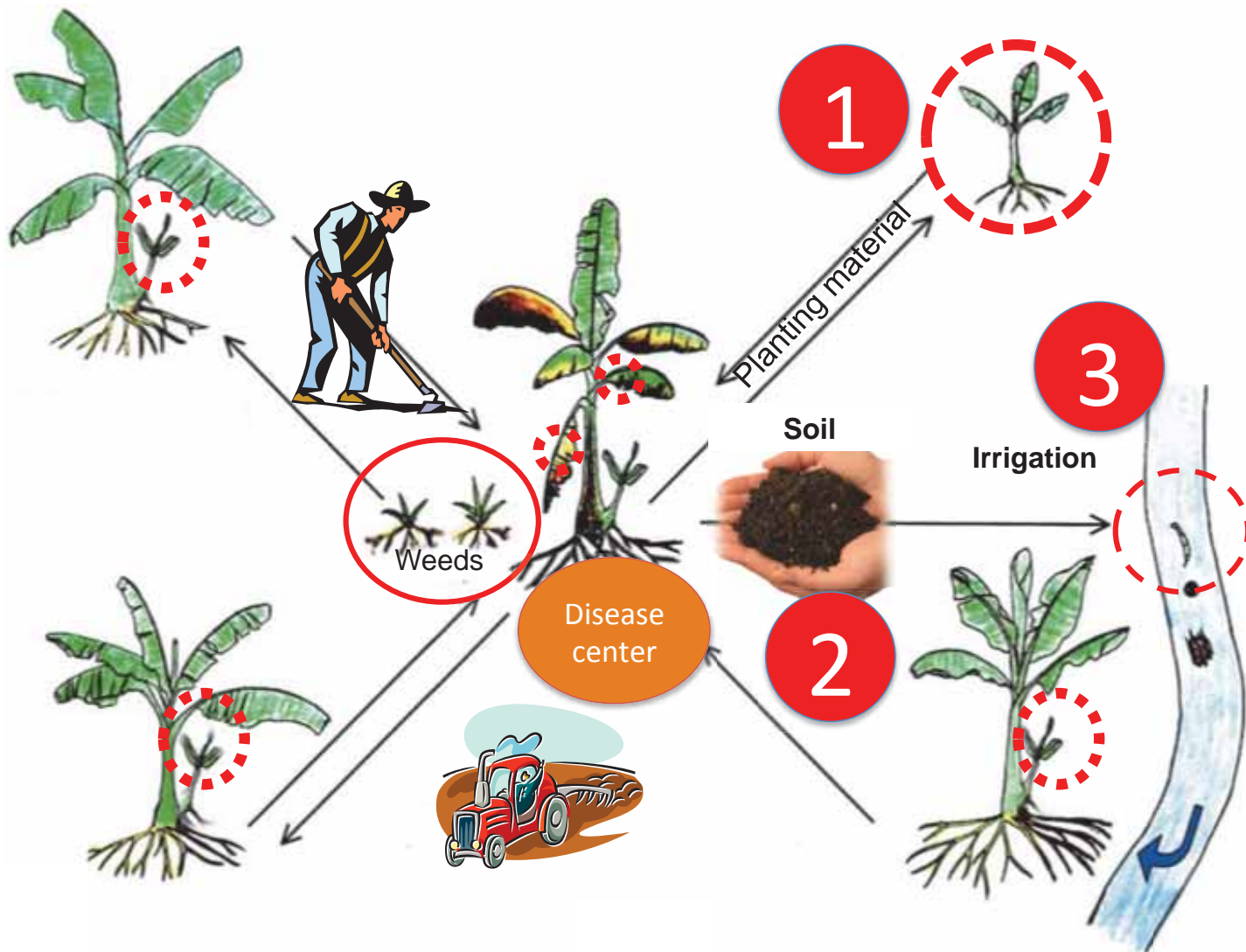
Benjin Li • Jialiang Du • Chengzhong Lan •
Peiqing Liu • Qiyong Weng • Qinghe Chen

Foc TR4 - *in planta* detection

Duplex PCRs

EF + FocTR4 // Actin2 + FocTR4





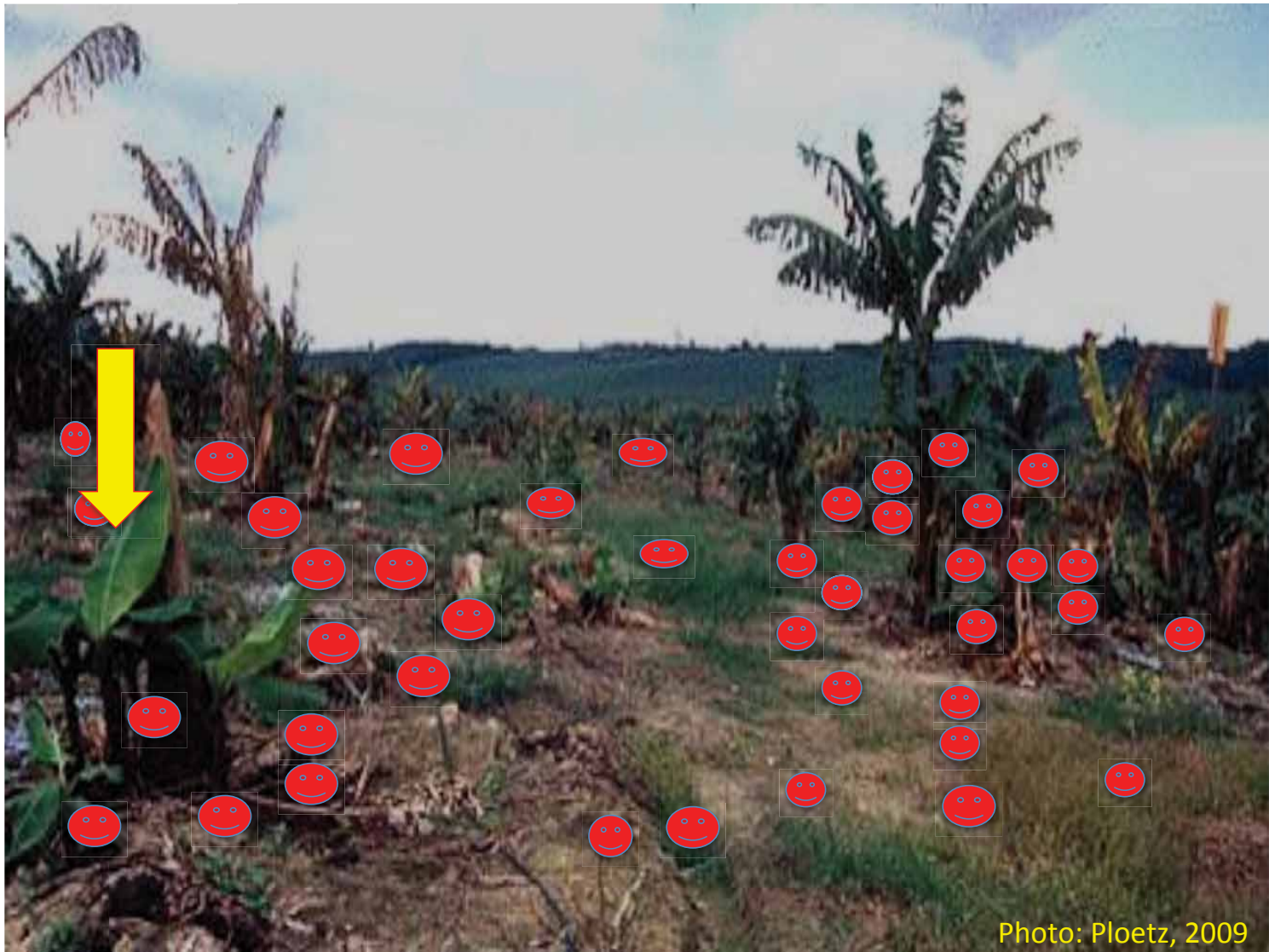


Photo: Ploetz, 2009



- Healthy plant ?
- Farmer leave this plant in the field or use suckers as planting material ?

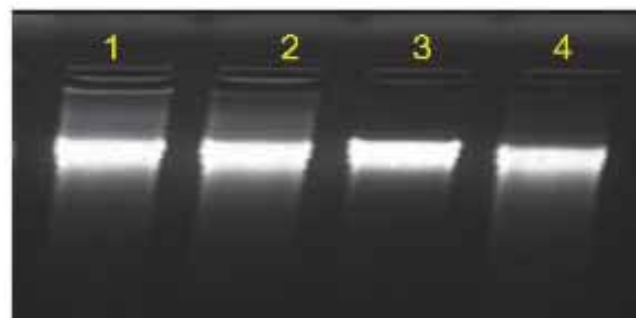
- Infected plant
- Farmer may not be aware about Foc infestation and use (even sell) symptomless, but infected suckers



Detecting Foc TR4 in soil and symptomless tissues

Sample	Host	Country	Plant stage
TS1	Cavendish	Taiwan	Healthy plant
TS2	Cavendish	Taiwan	Healthy plant
TS3	Cavendish	Taiwan	Infected plant
TS4	Cavendish	Taiwan	Healthy plant
TS5	Cavendish	Taiwan	Healthy plant
TS6	Cavendish	Taiwan	Healthy plant
TS7	Cavendish	Taiwan	Infected plant
TS8	Cavendish	Taiwan	Healthy plant
TWS9	Cavendish	Taiwan	Infected plant
AuD1	Cavendish	Austrália	Infected plant
AuD2	Cavendish	Austrália	Infected plant
AuD3	Cavendish	Austrália	Infected plant
AuH1	Cavendish	Austrália	Healthy plant
AuH2	Cavendish	Austrália	Healthy plant
AuD3	Cavendish	Austrália	Healthy plant
SEcu01	Cavendish	Equador	Healthy plant
SEcu02	Cavendish	Equador	Healthy plant
SCR01	Gros Michel	Costa Rica	Healthy plant
SCR02	Gros Michel	Costa Rica	Healthy plant

➔
DNA extraction



Detecting Foc TR4 in soil and symptomless tissues

Sample ID	Cultivar ^a	Plant stage	Location	Source	Sample processed	Single PCR	Nested PCR
AuD1	Cavendish	Symptomatic	Darwin	Australia	Plant	+	nd
AuH1	Cavendish	Symptomless	Darwin	Australia	Plant	+	nd
TS1	Cavendish	Symptomless	Chaozhou	Taiwan	Soil	-	+
					Plant	+	nd
TS3	Cavendish	Symptomatic	Chaozhou	Taiwan	Soil	+	+
					Plant	+	nd
TS6	Cavendish	Symptomless	Wandan	Taiwan	Soil	-	-
					Plant	-	nd
TS7	Cavendish	Symptomatic	Jiuru	Taiwan	Soil	+	+
					Plant	+	nd
TS8	Cavendish	Symptomless	Jiuru	Taiwan	Soil	-	+
					Plant	+	nd
TS9	Cavendish	Symptomatic	Luye	Taiwan	Soil	+	+
					Plant	+	nd
Phi126C	Gran Naine	Symptomatic	Kapalong	Philippines	Plant	+	nd
Phi39B	Tall William	Symptomatic	Kapalong	Philippines	Plant	-	nd
Phi2SV	Latundan	Symptomatic	Kapalong	Philippines	Plant	-	nd
ChlamyD2	n.a.	n.a.	n.a.	n.a.	Foc-colonized Substrate	+	+

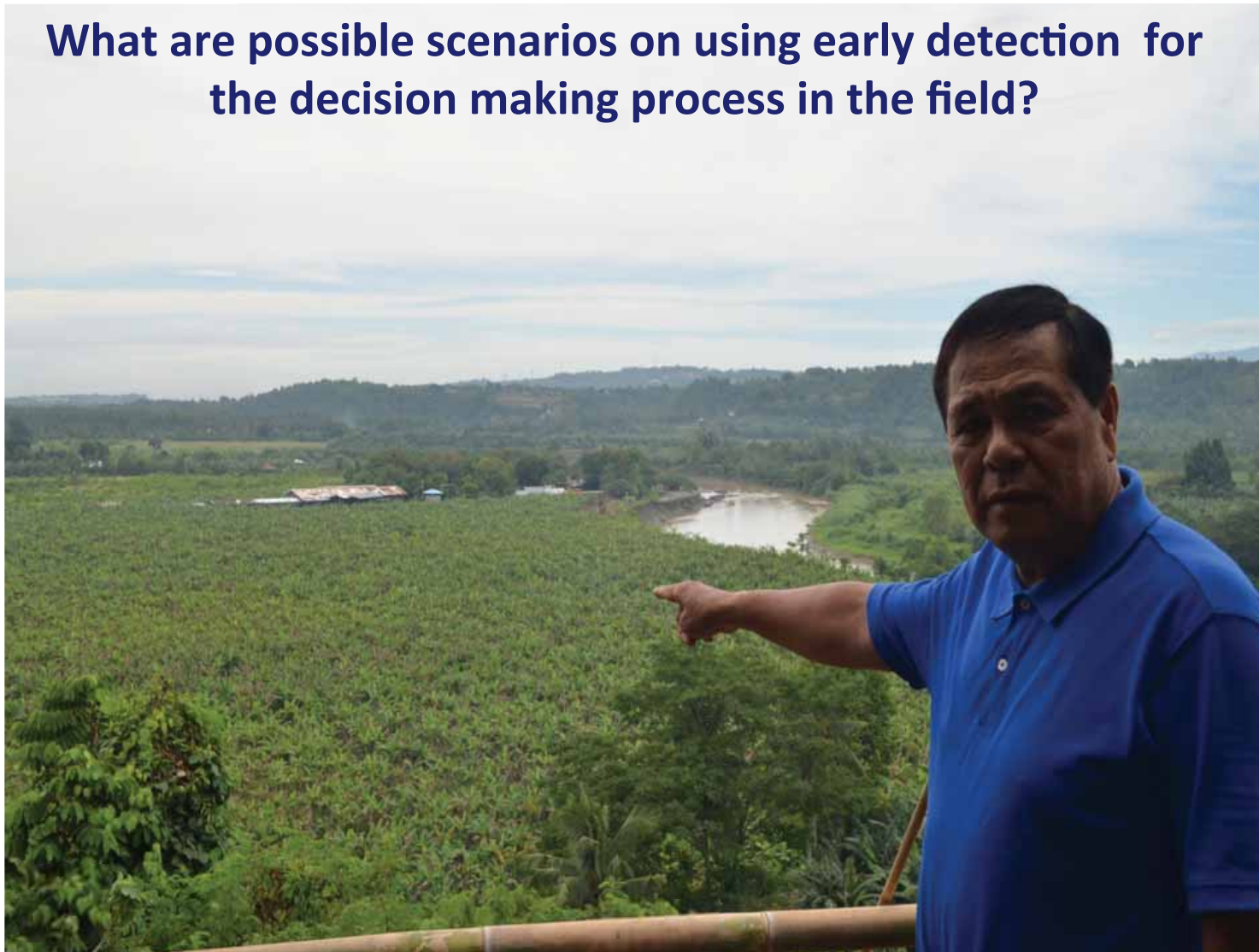
Soil DNA samples from Ecuador and Costa Rica have been consistently negative

Detecting Foc TR4 in soil



- ? – DNA quality*
- – Sample
- + sample

What are possible scenarios on using early detection for the decision making process in the field?



At this moment is to late: Early detection + eradication



Dissemination of TR4 in Cavendish plantation



According to Horticulture Plant Protection Department (2007) reported that epidemic rates of Foc race 4 in Sumatra and other provinces -

reach 100 km/year

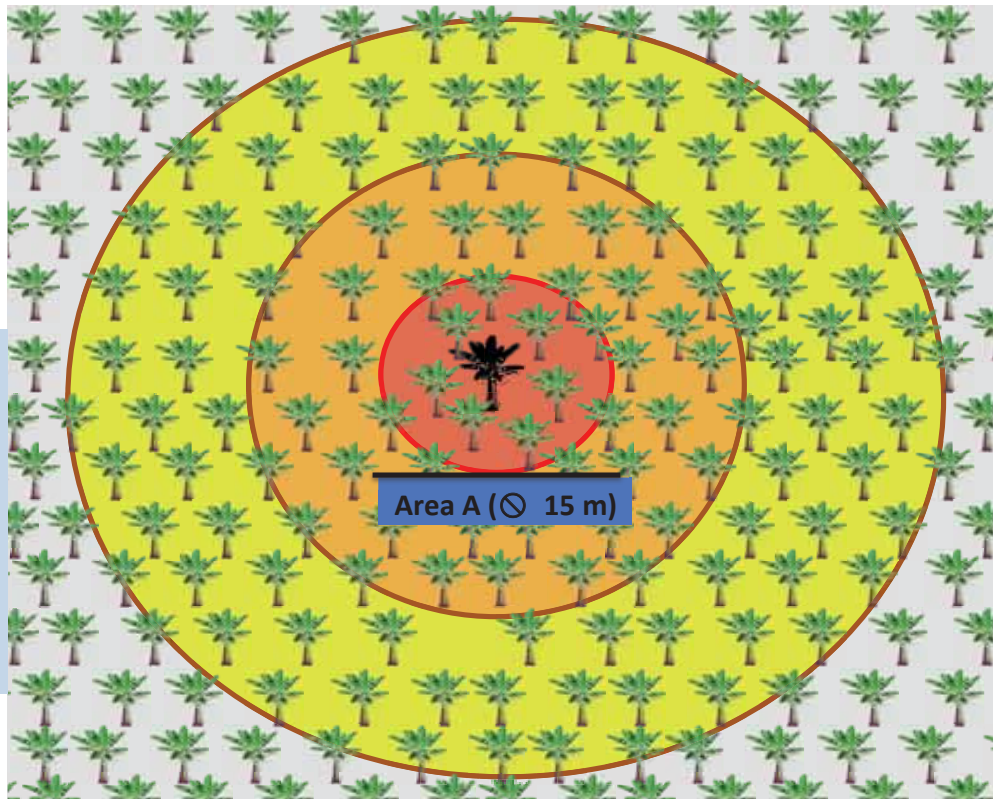




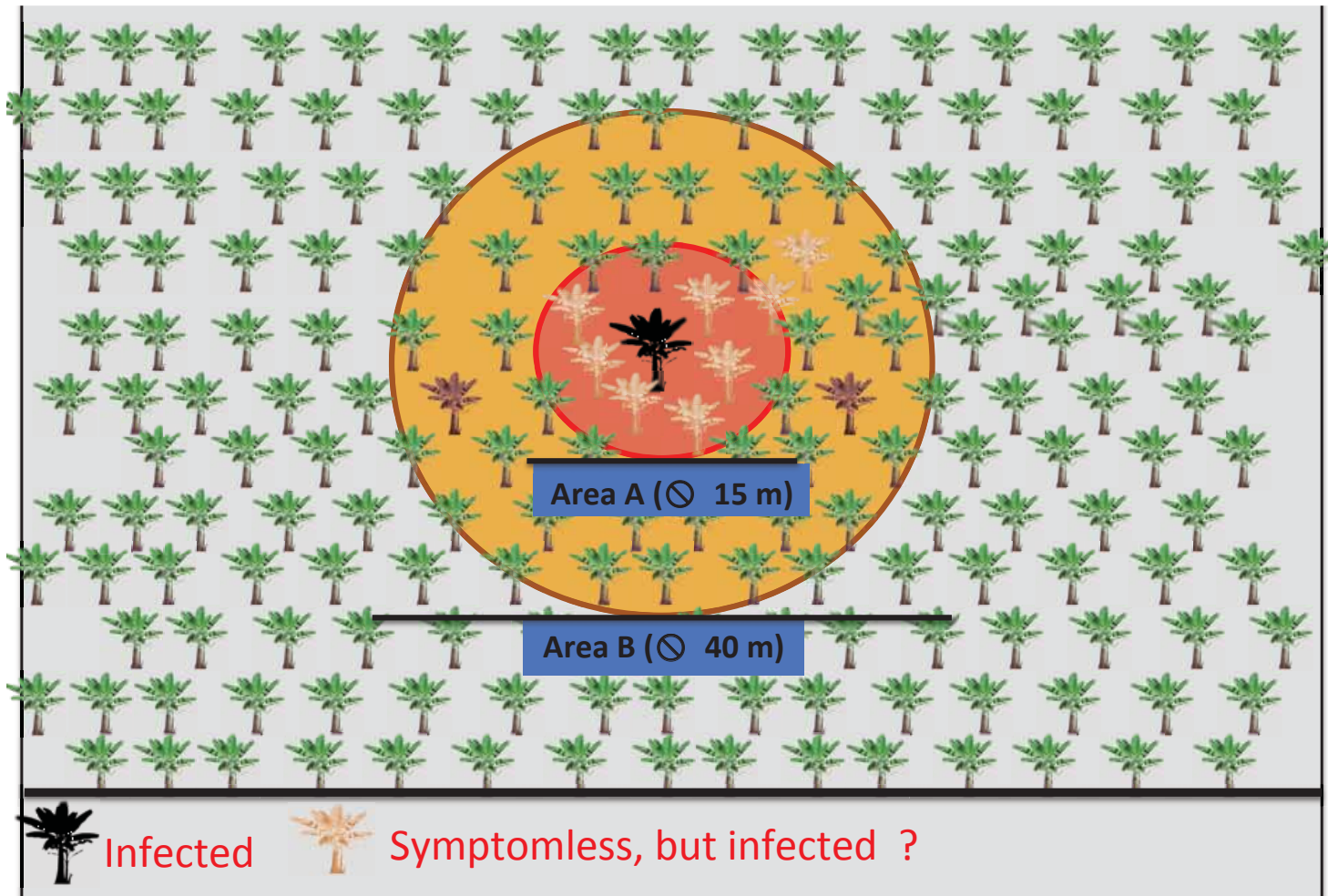
Early diagnostic of Fusarium wilt vs. management

When a plant is detected infected how many 'neighbors' we should destroy around?

Farmer and companies think more on bunches and boxes... they don't care too much on inoculum pressure, epidemiology ?
....but



Eradication



Aerial dissemination of Foc TR4?



Sporodochia formation
and external mycelia
growth

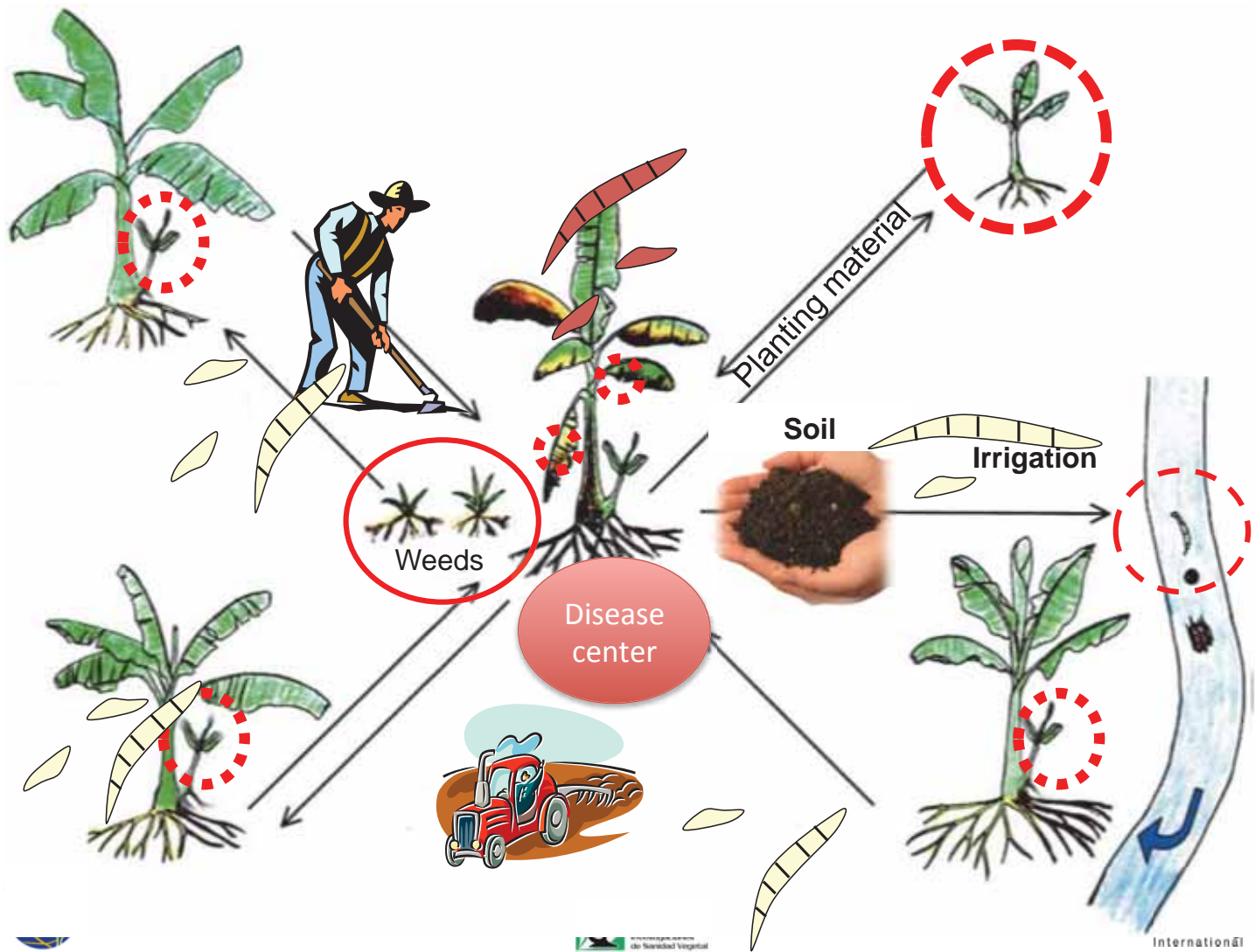


Photos: M.A. Dita



Foc RT4 en Cavendish/ R1
Gros Michel





Strengthening capacities and increasing preparedness for Foc TR4 in LAC

Importante

Fusariosis - Recomendaciones
para viajeros www.musalac.org



RECOMMENDATIONS TO PREVENT THE ENTRANCE OF QUARANTINE PLANT DISEASES OF MAJOR ECONOMIC IMPORTANCE FOR BANANA AND PINEAPPLE

Aimed at: Employees of the major fruit producing and processing companies, representatives of agrochemical companies and NGOs, certification inspectors, and any other visitor to banana plantations in South East Asia and pineapple plantations in South America

Risk Assessment and Surveillance of key banana diseases

Strengthening capacities and increasing preparedness for Foc TR4 in LAC

General Objective:

LAC countries strengthen capacities and increase preparedness on Fusarium wilt diagnostic and management aiming to prevent the entrance /early detect Foc TR4

Specific Objectives:

1. Government agencies, extension services and farmers **area aware** about the threat of quarantine pests of economic significance for the banana sector.
2. Technicians are **able to recognize** potential suspicious plants infected with Foc TR4 and proceed with **adequate diagnostic tools**
3. Participants have basic elements of epidemiology and management of Fusarium wilt of banana including those related to a **contingency plan**



Risk Assessment and Surveillance of key banana diseases

Strengthening capacities and increasing preparedness for Foc TR4 in LAC

Bioversity,
MUSALAC
&
National Plant
Health
Organizations



Sampling & shipment – quarantine pathogen! Should not be done ‘as usual ‘



Fotos: Plutarco Echevoyen



**Sampling & shipment – quarantine pathogen!
Should not be done ‘as usual ‘**



Photo: P. Echevoyen



Shipment aiming *in plant* detection



photo: P. Echegoyen

Sampling & shipment – quarantine pathogen! Should not be done 'as usual'



photo: P. Echevoyen

Steps

Sampling
Pseudostem, petioles etc.
Place, cultivar, etc



Is this Foc TR4?

© Miguel Dita

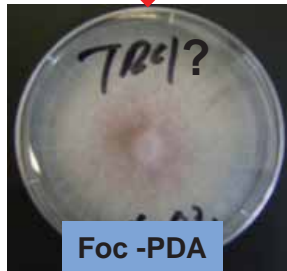
Sample shipment
from field to Lab



Sample processing



Foc isolation



DNA extraction



DNA extraction

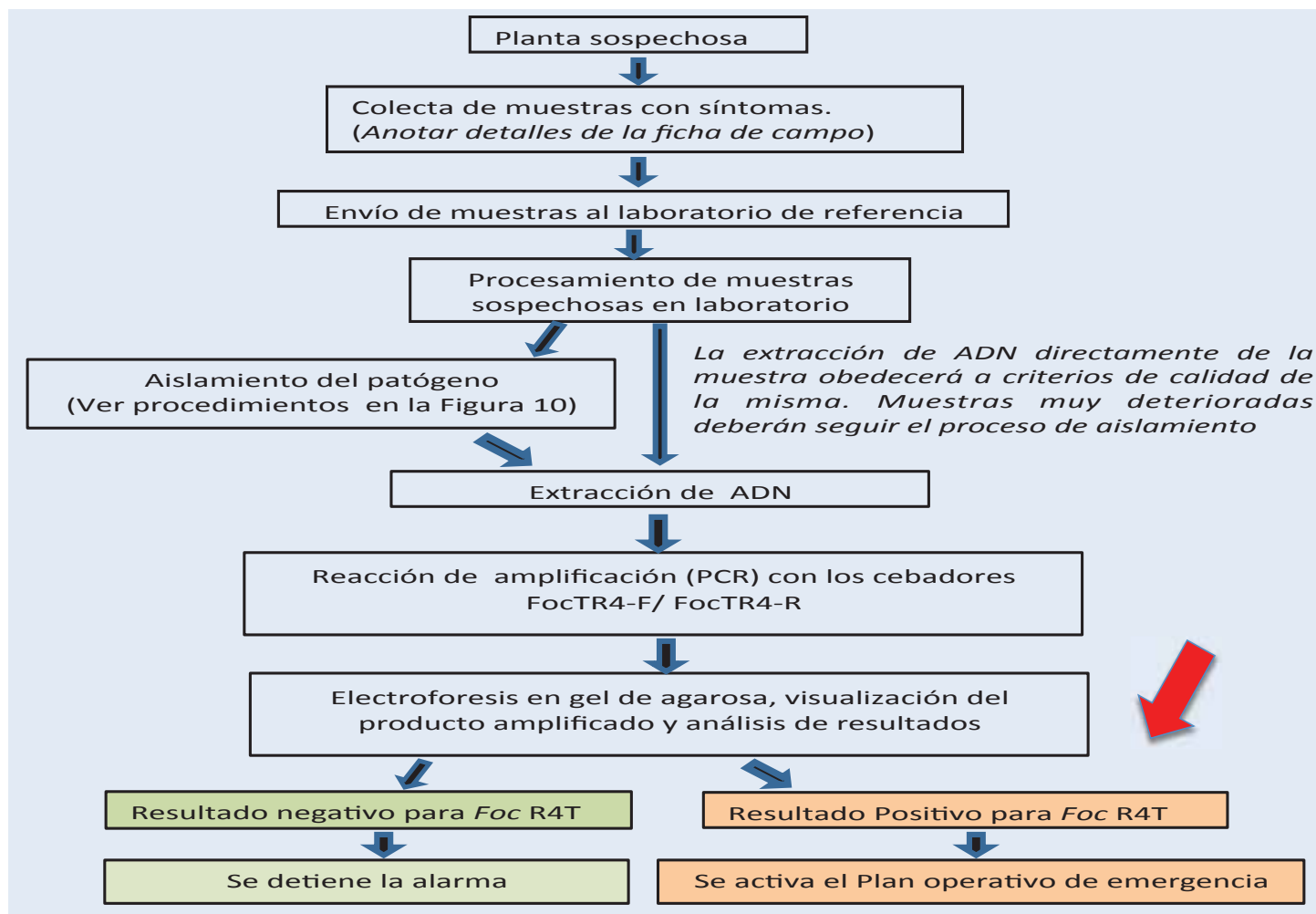


PCR

DNA extraction



General Instructed Steps at National Level



A Contingency Plan for Foc TR4

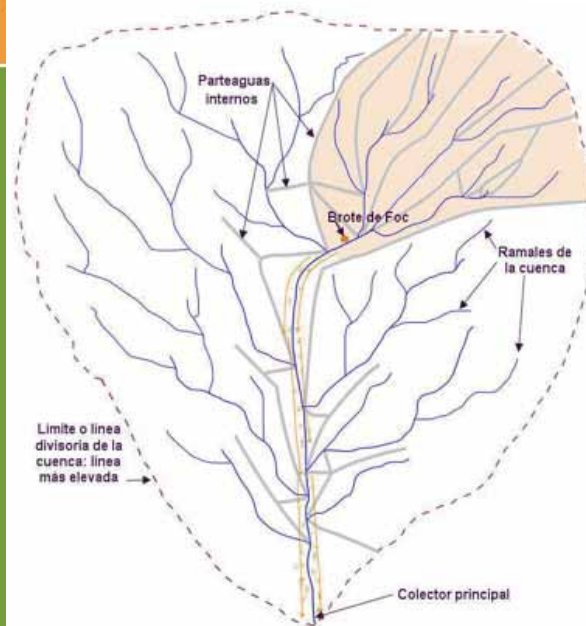
ORGANISMO INTERNACIONAL REGIONAL DE SANIDAD AGROPECUARIA
OIRSA

Plan de contingencia ante un brote de la raza 4 tropical de *Fusarium oxysporum* f. sp. *cubense*

En un país de la región del OIRSA



Elaborado por:
Miguel Ángel Dita Rodríguez
Plutarco Elías Echegoyén Ramos
y
Luis Fernando Pérez Vicente



Is glyphosate effective against Foc ?



Eradication & epidemiology Foc race 1

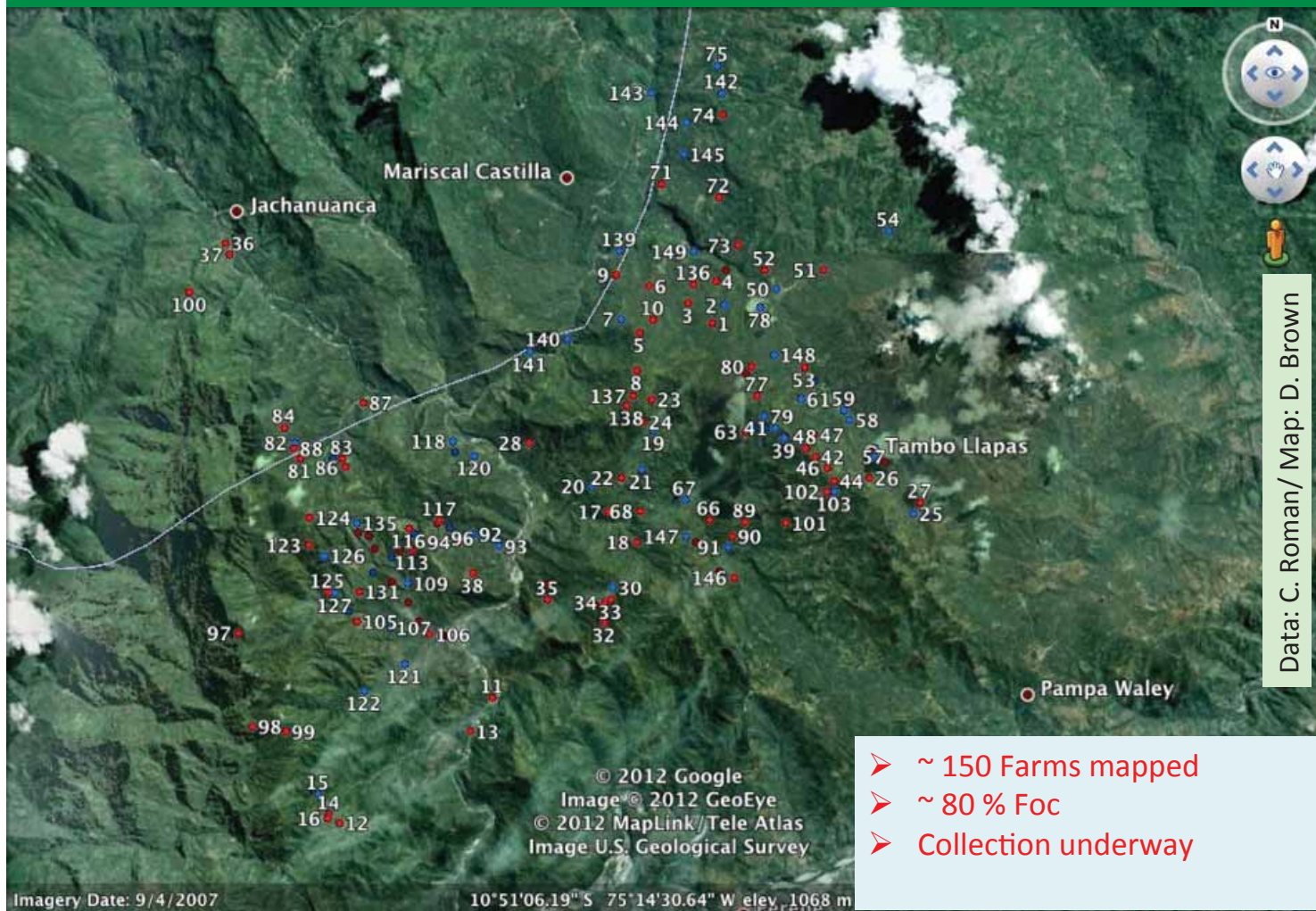
Is glyphosate effective
against Foc ?



Mapping and epidemiology of Foc in Peru



Epidemiology 4 management of Foc - Peru



Fusarium wilt: Integrated Management



Eradication of infected plantas



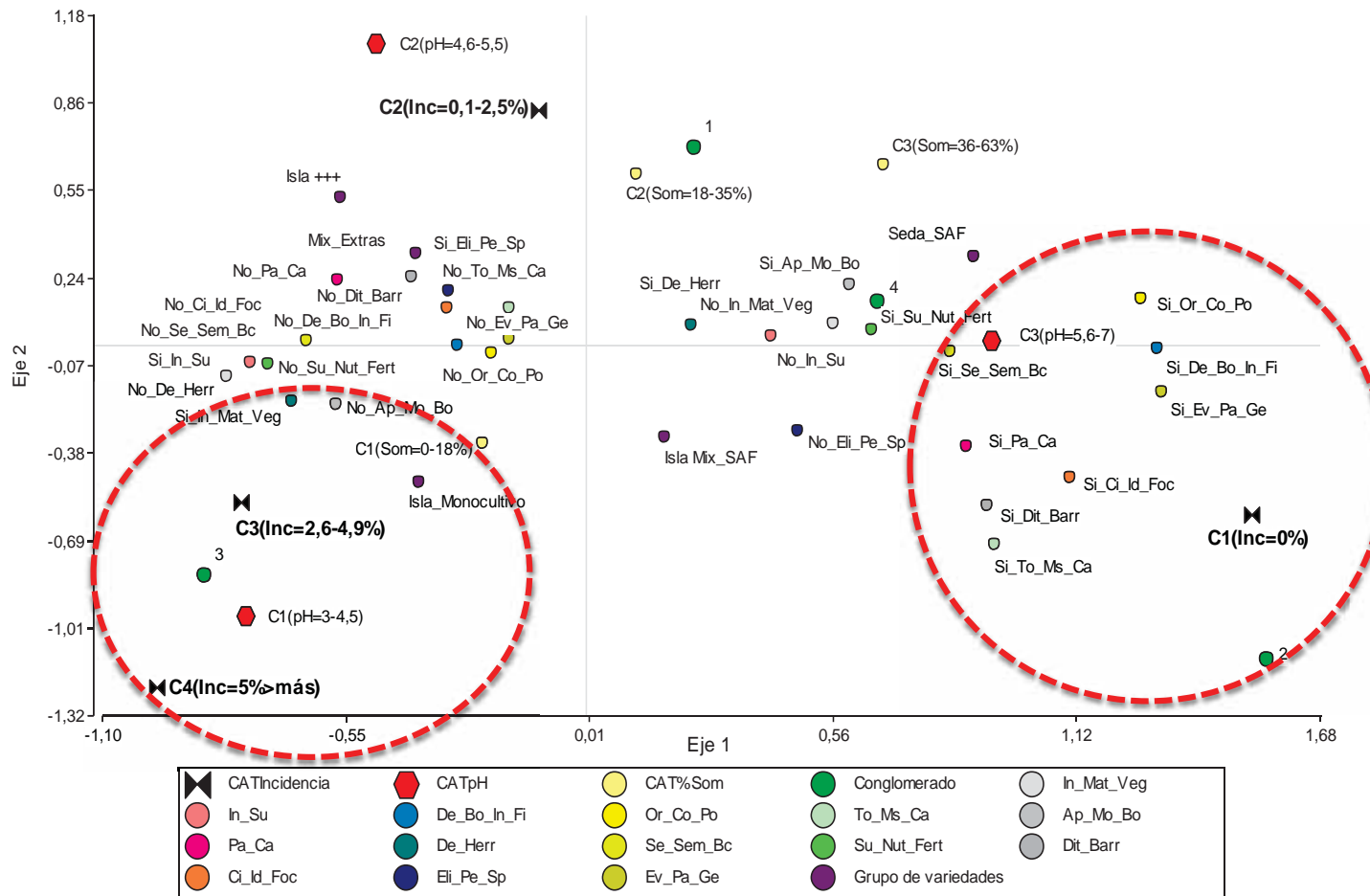
Tools desinfestation



Lemon?



Factors associated to Fusarium wilt intensity



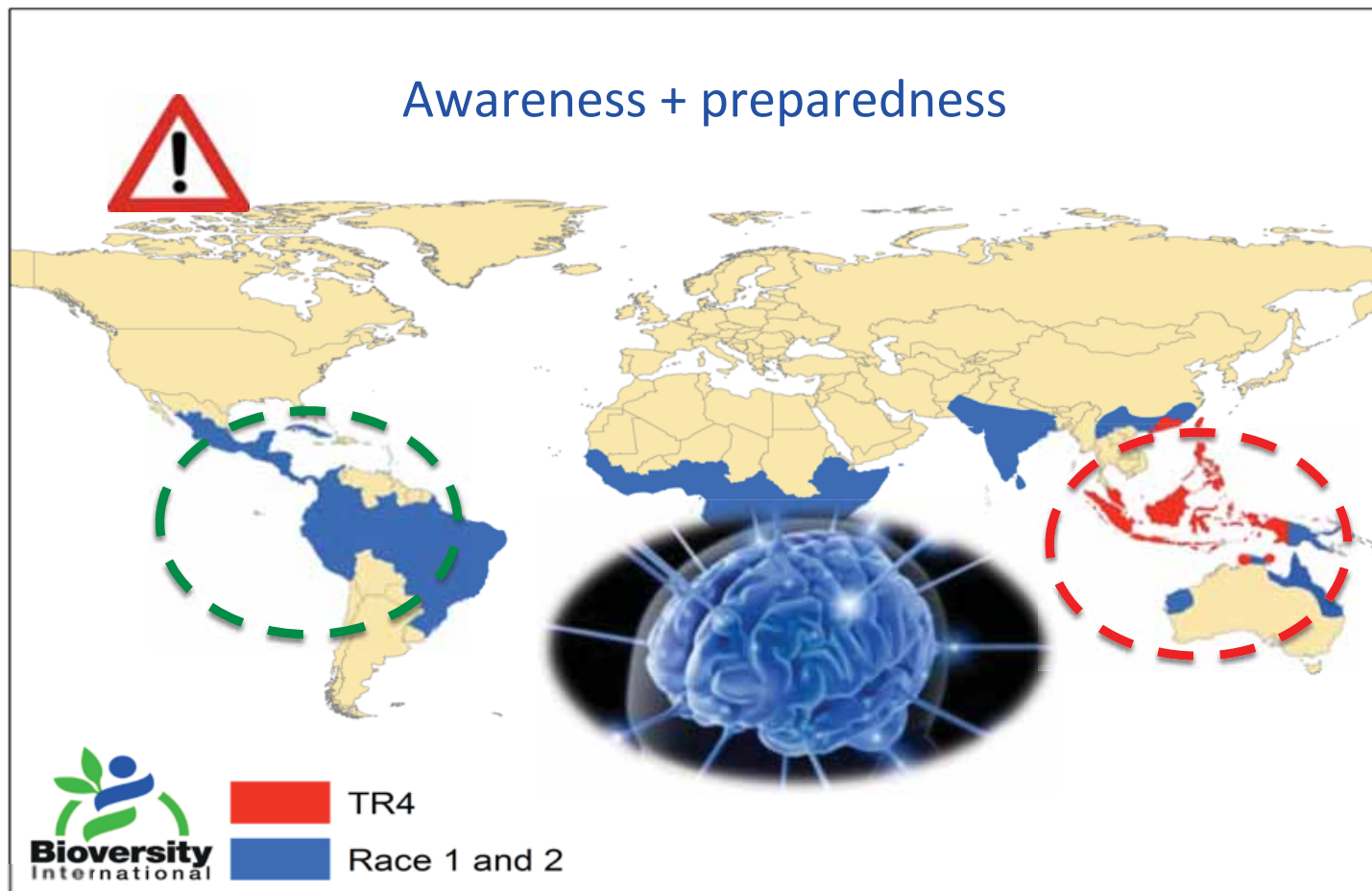
Red Lanitoamericana y el Caribe para la investigación y el Desarrollo de las Musáceas - MUSALAC,



Red Lanitoamericana y el Caribe para la investigación y el Desarrollo de las Musáceas - MUSALAC,

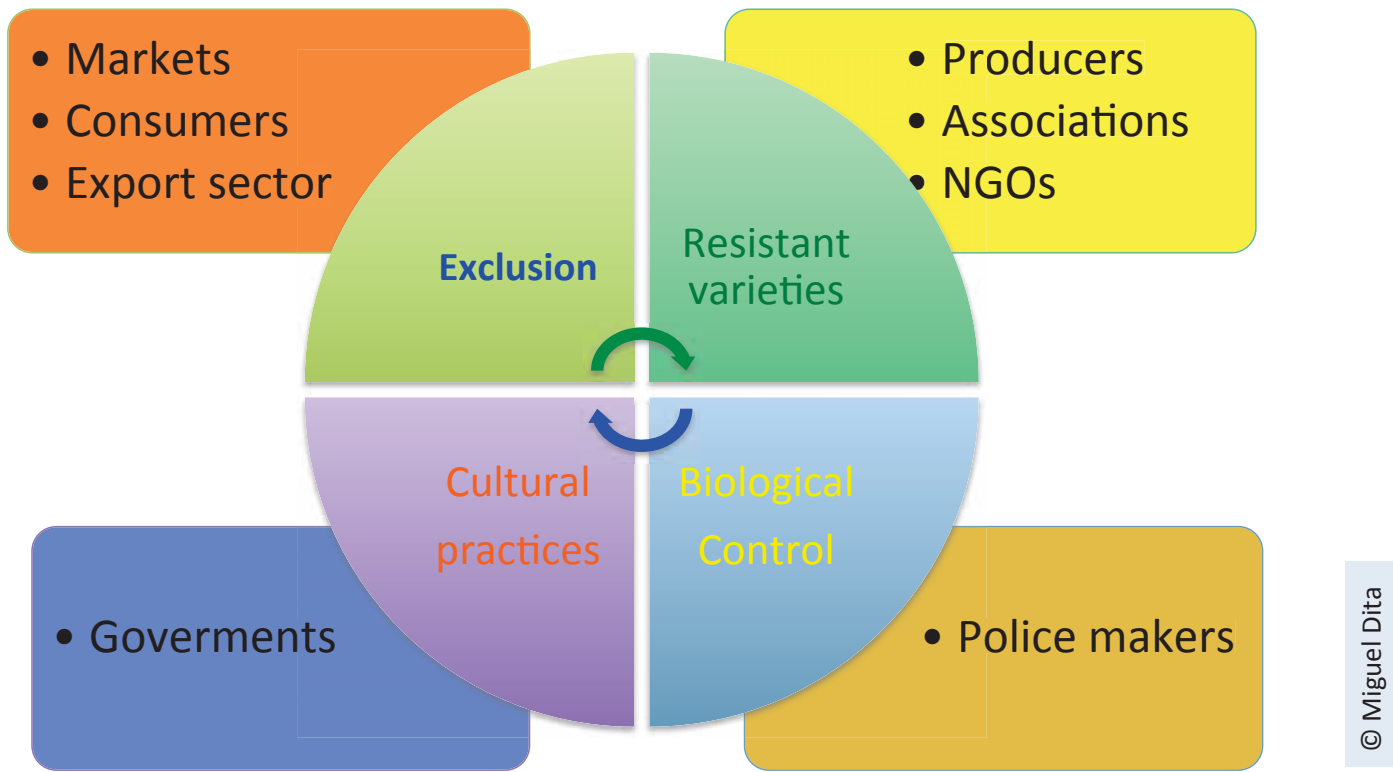


Fusarium Wilt of Banana: Cross-region collaboration



What would be the best scenario for TR4 prevention in LAC?

Integration!



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Collaborators

BIOVERSITY INTERNATIONAL

- Gus Molina



Cuba - INISAV

- Luis Pérez Vicente, Einar Martinez



Holland - PLANT RESEARCH INTERNATIONAL

- Gert Kema, Cees Waalwijk



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- P.F.L. Chang



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- B.M. Corcolon



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Gracias

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